

Claims:-

Sub 2

1. A method for characterising nucleic acid molecules, which comprises the steps of:
- 5 i) introducing a modified base which is a substrate for a DNA glycosylase into a DNA molecule;
- ii) excising the modified base by means of said DNA glycosylase so as to generate an abasic site;
- iii) cleaving the DNA at the abasic site so as to generate an upstream DNA fragment that can be extended; and
- 10 iv) incubating the extendible upstream fragment in the presence of an enzyme allowing for extension thereof and a template nucleic acid and analysing the resultant fragment(s).
2. A method according to Claim 1, wherein the upstream fragment is generated by cleaving the DNA at the 5' side of the abasic site, such that the 3' terminus of the upstream fragment bears a hydroxyl group.
- 15 3. A method according to Claim 2, wherein the cleavage is achieved with a 5' AP endonuclease.
4. A method according to Claim 1, wherein the upstream fragment is generated by cleaving at the 5' side of the abasic site so as to leave a phosphate group at the 3' terminus of the upstream fragment and removing the phosphate group so that the upstream fragment bears a hydroxyl group at the 3' terminus.
- 20

000201"6EZE950

5. A method according to Claim 1, wherein the upstream fragment is generated by cleaving at the 3' side of the abasic site so as to generate a deoxyribose phosphate group at the 3' terminus of the upstream fragment and subsequently removing the deoxyribose group to leave a hydroxyl group at the 3' terminus.

6. A method according to Claim 1, wherein 5' deoxyribose moieties downstream of the 3' terminus of the upstream fragment are removed so that the upstream fragment can be extended on the template.

7. A method according to Claim 6, wherein the 5' deoxyribose moieties are removed by a 5' deoxyribophosphodiesterase.

8. A method according to Claim 1, wherein the modified base is introduced by enzymatic amplification of the DNA.

9. A method according to Claim 8, wherein the amplified strands are separated for a separate analysis of the respective strands.

10. A method according to Claim 8 or 9, wherein a primer or one or more nucleotide(s) involved in the enzymatic amplification is labelled.

11. A method according to Claim 1, wherein the enzyme is a polymerase.

12. A method according to Claim 11, wherein the extendible upstream fragment is incubated with the polymerase in the presence of one or more nucleotide(s).

13. A method according to Claim 12, wherein one or more of the nucleotide(s) of step iv) is a dideoxy nucleotide.

A 14. A method according to Claim 12 ~~or 13~~, wherein one or more of the nucleotide(s) of step iv) is labelled.

A 15. A method according to ~~any one of Claims 11-14~~, wherein the extension of step iv) is achieved by means of an amplification reaction using said extendible DNA fragment.

A 16. A method according to ~~any one of Claims 11-15~~, wherein the extension of step iv) is achieved by means of an amplification reaction including a primer in addition to using said extendible DNA fragment.

10 17. A method according to ~~any one of Claims 1-10~~, wherein the enzyme is a ligase.

18. A method according to Claim 17, wherein the extendible upstream fragment is incubated with the ligase in the presence of a reporter oligonucleotide.

15 19. A method according to Claim 18, wherein the reporter oligonucleotide is partially degenerate.

A 20. A method according to ~~any preceding claim~~ <sup>Claim 1</sup>, wherein any extended fragments resulting from step iv) are detected by hybridisation.

A 21. A method according to ~~any preceding claim~~ <sup>Claim 1</sup>, which is used to detect a known or unknown mutation.

20 22. A method according to ~~any preceding claim~~ <sup>Claim 1</sup> for characterising nucleic acid molecules, substantially as hereinbefore described and exemplified.

A 23. A method according to ~~any one of Claims 1-20~~, wherein the method is used to analyse the CpG content of DNA by detecting C to T transitions in DNA.

~~24. A method according to Claim 1 for characterising nucleic acid molecules, substantially as hereinbefore described and exemplified.~~

000201" 6EZE960

Add D1